

**REMARKS**

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow. With this amendment, claims 95-103 and 113-120 have been amended, no claims have been cancelled, and claims 124-127 have been added. A detailed listing of all claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented, with an appropriate defined status identifier. Thus, claims 1-6, 10-12, 33-34, and 94-127 are pending in the application. Support for new claims 124-127 can be found in at least [0037] and [0047] of the specification. No new matter has been added.

**Claim Objections**

Claims 95 and 96 were objected to for minor informalities. Applicants respectfully traverse this objection.

Claim 95 has been amended to change “electrophoresis” in line 4 to “magnetophoresis.” Claim 96 has been amended to delete the second occurrence of “magnetophoresis.” Applicants submits that the minor informalities have been corrected and respectfully requests withdrawal of the objection.

In addition, the preambles of claims 97-103 and 113-120 have been amended to be consistent with the claims from which they depend. No new matter has been added.

**Claim Rejections -35 USC § 112**

Claims 1-6, 10-12, 33, 34, 94-120 were rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. Specifically, the Examiner alleges that the phrase “wherein the gel matrix has a thickness sufficient to perform electrophoresis” is new matter. Applicants respectfully traverse this rejection.

If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met. See, e.g., *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563 (Fed. Cir. 1991); *Martin v. Johnson*, 454 F.2d 746, 751, (CCPA 1972)(stating “the description need not be in *ipsis verbis* [i.e., “in the same words”] to be sufficient”). (See also MPEP 2163(II)(3)(a)). Description of a gel matrix thick enough to perform electrophoresis can be found throughout the application. Some examples include, “In one aspect, at least one of the nanoparticles contained in the gel matrix can have a net charge to aid in analyte separation during electrophoresis (paragraph [0030] of printed pub.),” “In another embodiment, the invention provides methods for detecting an analyte in a sample comprising contacting a sample containing an analyte with a separation gel … separating the complexes from other sample contents by electrophoresis or magnetophoresis. (*Id.* at [0035]),” “Separation of the complexes within the gel matrix is accomplished by electrophoresis or magnetophoresis. (*Id.* at [0037]),” “In addition, each probe construct in the set is specifically designed to have a unique mobility in the chosen electrophoresis medium. (*Id.* at [0047]).” Simply, one of ordinary skill in the art reading the specification would have understood that the disclosed gels are designed for electrophoresis or magnetophoresis and therefore are thick enough to perform electrophoresis or magnetophoresis. Applicants respectfully request withdrawal of the rejection.

### **Claim Rejections: 35 USC § 103**

Claims 1,2,5,10,33,94-97,100,102,105,108,110,113, 116 and 118 were rejected under 35 U.S.C. 103(a) as being unpatentable over West et al. (US 6,699,724) in view of Renn et al. (US 3,875,044). Claims 3,4, 11, 12,34,98,99, 103, 104, 106, 107,111, 112,114,115,119and 120 were rejected under 35 U.S.C. 103(a) as being unpatentable over West et al. (US 6,699,724) in view of Renn et al. (US 3,875,044), as applied to claim 1, further in view of Schultz et al. (US 6,180,415). Claims 6, 101, 109 and 117 are rejected under 35 U.S.C. 103(a) as being unpatentable over West et al. (US 6,699,724) in view of Renn et al. (US 3,875,044), as applied to claim 1, further in view of Mirkin et al. (US 2003/0211488). Applicants respectfully traverse these rejections.

“Rejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness”. *KSR Int'l Co. v. Teleflex Inc.*, No. 04-1350, slip op. at 11 (U.S. April 30, 2007)(citing *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006)). Independent claims 1, 33, 123 include the feature “wherein the gel matrix has a thickness sufficient to perform electrophoresis.” Independent claims 95 and 96 include the feature “wherein the gel matrix has a thickness sufficient to perform magnetophoresis.” These features are neither taught nor suggested by the applied references.

In the office action, the examiner acknowledges that “West et al. fail to teach the alginate gel capable of moving the molecules by electrophoresis.” (Office action, p.4, l.12-13). To fill this gap, the Examiner cites Renn for teaching that hydrated gel of alginate can be used for electrophoresis. (Id. at l.14-16). The Examiner also argues “the hydrogel of West et al. is glucose permeable (col. 13, lines 49-53), which indicates that the gel is thick enough for a sample solution to flow through the gel. The gel is thick enough to provide flow of a sample containing molecules, and therefore the thickness of the gel is sufficient to perform electrophoresis.” (Office action, page 7, line 19 to page 8, line 1).

In the previous response, however, Applicant noted that the support layer of West was only 2-100 µm, while electrophoresis layers are typically between 1 and 2 mm. Indeed, Applicant submit herewith four additional references which teach that electrophoresis layers are typically between 0.4 and 1.5 mm. Simply, one of ordinary skill in the art would not understand West to teach a layer suitable for electrophoresis. The gel of West, in contrast to the claimed electrophoresis gel, is only used as a support (col.3, l.48-51) and to prevent migration of the nanoshells (col.12, l.56-59). There is simply no rational basis to substitute the thin gel support layer of West with the thick electrophoresis gel of Renn. Further, Schultz et al and Mirkin et al. also fail to provide a rational basis for substituting the thin gel support layer of West with a thick electrophoresis gel. Applicants therefore respectfully request withdrawal of the rejections.

**New claims**

New claims 123-127 are drawn to the embodiment described in paragraphs [0037]-[0047] of the specification. In contrast with West, the nanoparticles of claims 123-127 are not stationary. Indeed, the different mobilities of the analyte-nanoparticles complexes of the claimed embodiment aid in separation and analysis. For at least this reason, new claims 123-127 are distinguishable over the applied references.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

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- Reference Attachments

## A versatile gel casting cum electrophoresis apparatus\*

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**Abstract.** A simple apparatus for vertical, *in situ*, polyacrylamide or agarose gel casting as well as for the subsequent electrophoresis is described. The apparatus is completely leakproof and does not require any special device like clamps, O-rings, gaskets, grease etc. for sealing. Slab gels of various thickness (0·04 to 1·0 cm) can be made and the apparatus can be used for analytical or preparative purposes. Gel rods can also be cast and run in the device. Forward as well as reverse polarity electrophoresis of a sample can be run simultaneously in the apparatus.

**Keywords.** Polyacrylamide gel electrophoresis apparatus; gel electrophoresis simple apparatus; gel casting cum electrophoresis; simultaneous forward and reverse electrophoresis.

### Introduction

Several models of vertical gel electrophoresis have been developed by different workers. Sophisticated commercial models are also available. In a recent comprehensive review on preparative gel electrophoresis, Chrambach and Nguyen (1979) have stressed the need for a simpler design for preparative gel electrophoresis which is free from the effects of mechanical and hydrostatic pressures on the gel. The equipment available including the commercial models possesses one or more of the following disadvantages: The gels are made separately in gel casting device and then transferred to the electrophoresis apparatus, thus developing mechanical stresses (Tichy, 1966; Akroyd, 1967; Studier, 1973; Bambeck and Black, 1981). Hydrostatic equilibrium is not attained (Tichy, 1966; Akroyd, 1967; Studier, 1973; Ogito and Market, 1979). Moreover to avoid leakage, the above mentioned models and also those described by Blatter (1969), Roberts and Jones (1972) and Andrew *et al.* (1979) require devices such as clamps, screws, O-rings, gaskets, melted agar, grease and plasticine clay.

This paper describes a simple and inexpensive, multipurpose apparatus for *in situ* gel making and subsequent electrophoresis which is free from the disadvantages mentioned above. An added advantage of this apparatus is that, with minor modifications, it

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Abbreviations used: PA, Polyacrylamide; PAGE, polyacrylamide gel electrophoresis; Bis, N,N'-methylenebis(acrylamide); TEMED, N,N,N',N'-tetramethylethylenediamine; UV, ultra-violet.

can also be used to obtain a total scan of biological extracts by running a simultaneous forward and reverse polarity electrophoresis.

## Materials and methods

### Chemicals

All common chemicals used were of the analytical reagent grade. The following chemicals and biochemicals were obtained from the sources indicated: myoglobin (from horse heart), trypsin (from bovine pancreas, Type III, EC 3.4.21.4), albumin (bovine serum, crystallized), amido black 10 B, ethidium bromide (crystalline) and ferritin (from horse spleen, Type I) were from Sigma Chemical Co., St. Louis, Missouri, USA; and casein (Hammersten) was from E. Merck, Dermstadt, West Germany. The chemicals used for polyacrylamide gel electrophoresis (PAGE) such as acrylamide, N,N'-methylenebis acrylamide (Bis) and N,N,N',N'-tetramethylenediamine (TEMED) were obtained from Eastman Kodak Company, Rochester, USA. Glycine was from Kochlight Laboratories, Buckinghamshire, UK. Agarose (electrophoresis grade) was purchased from Sisco Research Laboratories, Bombay. DNA marker (Hind III digest of  $\lambda$  DNA) was a generous gift from Dr. S. Modak, Poona University, Poona. Culture filtrate from the fungus *Conidiobolus* (Srinivasan *et al.*, 1983) was used for simultaneous forward and reverse electrophoresis. Perspex sheets were procured locally.

### Solutions for gel electrophoresis of proteins and nucleic acids

*Solutions for alkaline pH runs:* These were prepared according to Davis (1964). Both the upper and lower electrode buffers contained 0.005 M Tris and 0.04 M glycine, pH 8.3. The gel composition was 0.38 M Tris and 0.06 M HCl, pH 8.9; 7% acrylamide, 0.18% Bis, 0.03% TEMED and 0.07% ammonium persulphate.

*Solutions for electrophoresis at acidic pH values:* These were prepared according to Reisfeld *et al.* (1962) with slight modification. The two electrode buffers contained 0.04 M glycine and 0.0035 M acetic acid, pH 4.0. The gel composition was 0.36 M acetic acid and 0.06M KOH, pH 4.3, 7% acrylamide, 0.1% Bis and 0.14% ammonium persulphate.

*Solutions for agarose gel electrophoresis:* These were prepared according to Thomas and Davis (1975). Both the electrode buffers contained 0.089 M Tris, 0.089 M boric acid and 2.5 mM EDTA, pH 8.2. Agarose gel composition was 0.8% agarose in the above buffer.

*Tracking dye:* Basic fuchsin was used for acidic runs and bromophenol blue for other runs.

*Composition of contact gel:* Contact gel was made in the bath buffer composition of the respective runs (*e.g.* contact gel composition for alkaline runs was: 0.005 M Tris and 0.04 M glycine, pH 8.3, 7% acrylamide, 0.18% Bis, 0.03% TEMED and 0.07%

ammonium persulphate. Contact gel composition for the simultaneous forward and reverse runs was: 0·005 M KCl, 7% acrylamide, 0·18% Bis, 0·06% TEMED and 0·14% ammonium persulphate. Contact gel can be reused several times by preserving the gel in cold under a layer of the buffer used for making it.

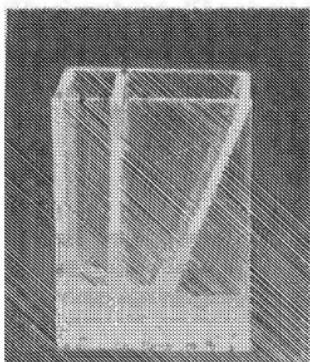
*Staining and destaining:* The gels were stained with amido black (0·5 % in 7% acetic acid) and destained with 7% acetic acid. Agarose gel was stained with ethidium bromide (1 mg/litre H<sub>2</sub>O) and the DNA markers were visualized over an ultra-violet (UV) transilluminator.

*Elution and assay of trypsin:* Trypsin was eluted from PAGE by the method of Bodhe *et al.* (1982) and estimated by the spectrophotometric assay of Kunitz using caseinolytic assay (Kunitz, 1947).

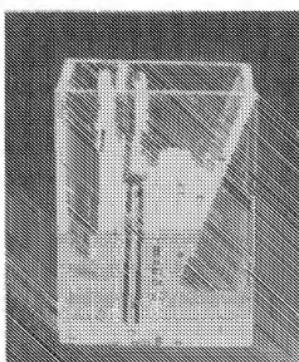
#### Apparatus

*Design principle:* The apparatus is designed for vertical gel electrophoresis. It is made from perspex sheets. Figures 1 and 2 show the photographs of the empty and the assembled unit. In addition figures 3 and 4 give the dimensions of the unit.

The main apparatus consists of two vertical chambers which form the electrode compartments. These chambers are adjacent to each other and are separated by a common middle partition wall. A gap of 0·8 cm is kept at the base of the partition wall. One cm thick gel is cast at the base of the chambers which also closes the gap (0·8 cm) kept below the partition wall. Thus this basal 'contact gel' makes the two chambers leakproof (prevents the buffer flow from one chamber to the other), and also establishes an electrical contact between the two chambers.



1



2

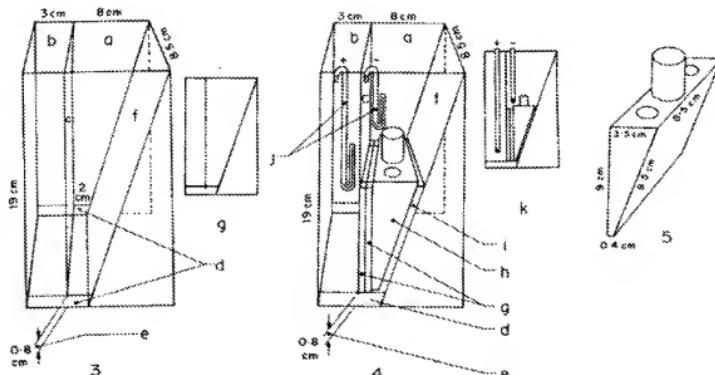
**Figures 1 and 2.** 1. Photograph of the empty unit. 2. Photograph of the assembled unit.

Gel cassettes (molds for casting slab gels) are placed above the contact gel in one of the chambers (chamber 'a') and are secured in position by a wedge (figures 4h and 5). Polyacrylamide (PA) slab gels are cast in the gel cassettes, both the chambers are filled with buffer and after loading the sample, *in situ* electrophoresis is carried out since both the chambers also form the two electrode compartments. Thus out of the two chambers one chamber serves as a chamber for gel casting and both the chambers act as electrode compartments.

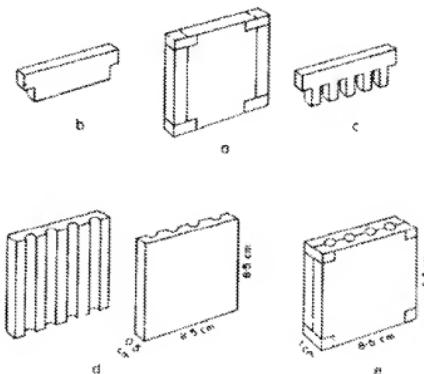
*Mode of construction:* In chamber 'a' where the gels are cast, the wall opposite the middle partition wall is made slanting for the operation of wedge. The middle partition wall is made of glass and sealed with araldite against the walls of the equipment.

The wedge (figures 4h and 5) is made of perspex sheets, except for its surface facing the gel cassettes which is made of glass and sealed to the wedge with araldite. The wedge is hollow and opens from the top only (see discussion).

Gel cassettes (glass, plates, 8.5×8.5 cm and 0.1 cm thickness) and well former for slab gel are made as in the Pharmacia apparatus (GE-2/4). Figure 6 shows the details regarding the slab and rod gel cassettes. For making a slab gel cassette, two spacer strips



**Figures 3-5.** 3. Empty Unit, (a) Chamber with slanting wall, with 2×8.5 cm base. Second chamber, 3×8.5×19 cm; (c) Common middle wall, 8.5×18.2 cm height; (d) Two support strips each 5.5×0.5×1 cm; (e) 0.8 cm gap below the common middle wall; (f) Slanting wall of chamber 'a'; (g) Elevation of the empty unit. [Thickness of the perspex sheet (0.5 cm) used in the construction of the main unit is not shown in the figure]. 4. Assembled Unit. (a) Chamber with slanting wall, with 2×8.5 cm base; (b) Second chamber, 3×8.5×19 cm; (c) Common middle wall, 8.5×18.2 cm height; (d) Two support strips, each 5.5×0.5×1 cm; (e) 0.8 cm gap below the common middle wall; (f) Slanting wall of chamber 'a'; (g) Gel cassettes; (h) Wedge; (i) Spacer or spacers if necessary, each 8.5×9.5 cm height, thickness from 0.3 to 0.5 cm; (j) Two 'L' shaped platinum electrodes. The electrode in the sample chamber 'a' is held above and closer to the surface of the gel cassettes. Similarly the electrode in the other chamber is held closer to the contact gel; (k) Elevation of the assembled unit. 5. Wedge (with two circular openings and a handle on the top).



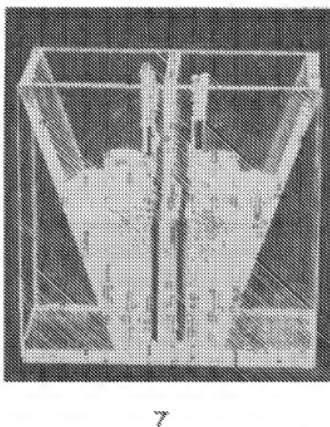
**Figure 6.** Gel cassettes, (a) Slab gel cassette; (b) Single well former for slab gel; (c) Multi well former for slab gel; (d) Two halves of the gel rod cassette (each 8.5×0.5×8.5 cm) exposing the half round grooves; (e) Gel rod cassette assembled, 8.5×1.0×8.5 cm.

are placed at the edges of a glass plate and the second glass plate is placed on them. The assembly is then held in position by two small strips of adhesive tape. The thickness of the gel can be varied as desired (0.04 to 1 cm) by using spacer strips of different thickness. For preparative type gel a 'single well former' is used. Spacers of different thickness and 'well formers' with different number of teeth can be easily cut from neoprene sheets.

Gel cassette for gel rod casting is made as shown in figure 6. It is made from two glass plates (8.5×0.5×8.5 cm) in which four half round groves of 0.3 cm radius are made on each plate. When both the plates are placed on one another four hollow tubes are formed inside. The rod 'well former' is made of cylindrical perspex teeth.

#### *Operating procedure*

Forty ml of 7% 'contact gel' is polymerised at the base of the apparatus. This fills up the base and the basal gap between the two chambers and comes up to a 1 cm height and thus to the level of the support strips. One or more slab gel cassettes are placed in chamber 'a' at the base of the apparatus on the two support strips and above the contact gel. The cassettes are secured by the wedge (figure 4h). After polymerising the gel in the cassettes, both the chambers of the apparatus are filled with the bath buffer, sample is loaded, electrodes are positioned and the run is started by making electrical contacts. After completing the run the bath buffer in the chambers is poured out and after removing the wedge the gel slabs are taken out, stained, destained or if desired the proteins can be electrophoretically eluted (Bodhe *et al.*, 1982). Gel rod is removed simply by opening the two halves of the rod casting cassette.



**Figure 7.** Photograph of the assembled unit for 'simultaneous forward and reverse electrophoresis'.

#### *Simultaneous forward and reverse polarity electrophoresis*

The above mentioned apparatus with a few modifications was used for simultaneous forward and reverse polarity electrophoresis. This design can be used for scanning biological extracts containing both acidic as well as basic proteins in a single run. The modification involves making chamber 'b' a replica of chamber 'a'. Consequently the unit will have two slanting walls and two wedges. Figure 7 shows the photograph of the assembled unit. In this apparatus the gels are cast in both the compartments (one gel in each compartment), and the same sample is loaded in both the compartments. Electrophoresis is carried out as mentioned in the earlier unit. Thus in this apparatus both the chambers serve as chambers for gel making as well as, as electrode compartments.

## Results

Electrophoresis of marker protein, trypsin, DNA markers and culture filtrate of *Conidiobolus*.

#### *Electrophoresis under alkaline conditions*

**Normal size gels:** Two slab gels (each 8.5×0.3×8.5 cm) were run together. In one gel a mixture of 400 µg each of ferritin, myoglobin, serum albumin and trypsin inhibitor were loaded. In the second gel the same marker mixture was loaded in three wells (15,30

and 45 µg of each protein respectively). The band pattern of the proteins stained is shown in figure 8.

*Thin gels:* Three gels (8.5×0.04×8.5 cm) were run simultaneously in the apparatus. Fifteen per cent gels were made instead of 7%. In each gel, marker protein mixture (30 µg of each marker) was loaded. After electrophoresis, gels were stained for 5 min in amido black and destained in 10 min by suspending in 7% acetic acid (figure 9).

*Thick gel:* Preparative run in 1 cm thick gel. One gel (8.5×1.0×8.5 cm) was run. Marker mixture containing 7 mg of each marker protein (total 28 mg protein) was electrophoresed (figure 10).

*Gel rods of high PA concentration:* Four gel rods of 15% PA were cast in the cassette and a mixture of 20 to 40 µg of each marker protein was loaded in each gel rod. After the run the rods could be easily taken out by simply opening the cassette (figure 11).

#### *Electrophoresis under acidic conditions*

*A labile protein:* Trypsin was run in a cold room at 5 to 8°C. Two slab gels (8.5×0.3×8.5 cm) were run together. Two mg of trypsin was loaded in each gel. After the run, one gel slab was stained (figure 12) and the other gel was used for the electrophoretic elution of trypsin (Bodhe *et al.*, 1982), 78% of the trypsin activity was eluted.

#### *DNA markers in agarose gel*

One gel slab (8.5×0.3×8.5 cm) containing 0.8% agarose gel with wells was run after loading 0.1 µg of DNA marker (figure 13).

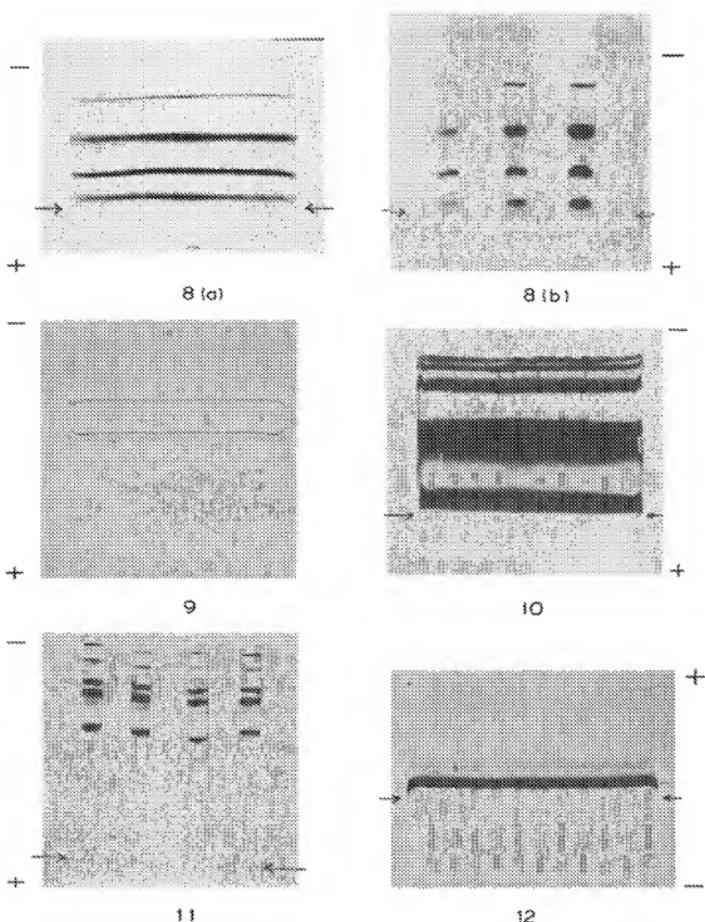
#### *Simultaneous forward and reverse polarity electrophoresis of fungal broth*

*Both forward and reverse runs at pH 8.9:* The gel and buffer compositions in both the chambers were those of pH 8.9 system. Broth sample (5 mg protein) was loaded in the gel of each chamber. Figure 14 shows the protein band pattern.

*Forward run at pH 8.9 and reverse run at pH 4.3:* In one chamber the gel and the buffer composition was that of pH 8.9 system. In the other chamber the gel and buffer composition was that of pH 4.3 system. Broth sample (5 mg protein) was loaded in the gel of each chamber. The band patterns of the stained gels is shown in figure 15.

## **Discussion**

We report here a dual purpose simple and leakproof apparatus for vertical PA or agarose gel preparation and for the subsequent electrophoresis. Slab gels, as well as gel rods can be cast and run in the apparatus. Gel slabs of various thickness (0.04 cm to 1 cm) can be made and thus the apparatus can be used for analytical as well as for



**Figures 8–12.** 8. Two normal size gel slabs run together, each  $8.5 \times 0.3 \times 8.5$  cm. (a) Protein markers (ferritin, myoglobin, serum albumin and trypsin inhibitor), each 400 µg. Position of the tracking dye is shown with arrows; (b) Protein markers in three wells; 15, 30, 45 µg of each marker was loaded. Electrophoresis was at 90V, 25mA for 3 h. 9. Three thin gel slabs together, each  $8.5 \times 0.04 \times 8.5$  cm. Protein marker mixture containing 30 µg of each marker

preparative purpose. More than one gel can be run in the apparatus. Gel rod cassette eliminates the troublesome removal of gels from the tubes. This is especially convenient for the gels of 15% or higher PA concentrations and does not require any rimming or tube breaking.

Hydrostatic balance (Chrumbach and Nguyen, 1979) is automatically adjusted due to the position of the two buffer chambers. Mechanical stress (Chrumbach and Nguyen, 1979) on the gel is also avoided by *in situ* polymerization of the gel and subsequent electrophoresis in the same apparatus. Contact gel is reusable. The band patterns obtained in the device described are as good as those obtained in other models.

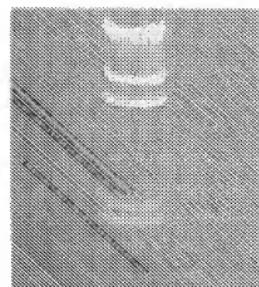
The apparatus shows some resemblance with the Pharmacia gel making apparatus (Pharmacia Fine Chemicals, Sweden, Gel Slab Casting Apparatus GSC-8). However, the introduction of one of the main vital modifications—the gap kept below the central partition in our apparatus has made a vast difference and made the unit a 'two in one' unit *i.e.* the same apparatus is used for gel casting and subsequent electrophoresis also, for which M/s. Pharmacia had to develop a separate and costly electrophoresis apparatus. In our device the gel thickness can be varied from 0.4 mm to 10 mm, which is not possible in the Pharmacia electrophoresis apparatus. In the gel rod cassette of the present apparatus, gels of very high acrylamide concentration (30 to 40 % acrylamide) can be cast and the gel rods after the run can be easily removed from the cassette by just opening the two halves of the cassette like a book. Removal of high concentration gel rods is not possible in the commercial models. High concentration gel rods can be used for electrophoresing small molecular weight proteins and peptides. The present apparatus has also other advantages as mentioned earlier in 'Introduction'.

The modified device (figure 7) can be used for a simultaneous forward and reverse polarity electrophoresis of a sample. Thus in a single run it will be possible to get a total protein pattern of a sample. In most of the reported models this facility is not available. This modified device can also be used for usual electrophoresis (unidirectional) as in our normal device (figure 2). However, the normal device is more compact, cheaper and easy to fabricate as compared to the modified design. Although simultaneous forward and reverse electrophoresis can be carried out only in the modified device, for routine runs the compact device is quite satisfactory.

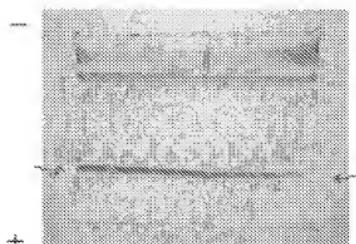
Under normal experimental conditions the problem of heat dissipation—elimination of heat generated in the gels, is well taken care of in our device. Even during the preparative run the temperature rise in the bath buffer and in the gel proper, is not more than 3°C and 7°C respectively. In the case of labile proteins, runs can be carried out in a cold room. Since glass is a better conductor of heat than perspex, one surface of the wedge facing the cassette and the middle partition wall between chamber 'a' and 'b' are made of glass. When chamber 'a' is filled with bath buffer the hollow wedge also gets filled with it which cools the gel through the glass surface. Also the glass wall of middle partition offers cooling by the bath buffer of chamber 'b'.

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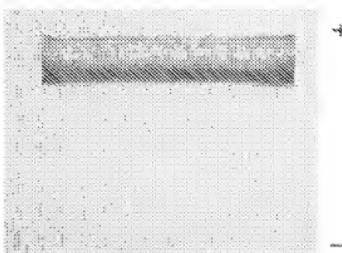
was loaded. Electrophoresis at 100 V, 12 mA for 2 h. **10.** One thick gel, 8.5×1×8.5 cm; preparative scale. Marker mixture, 7 mg of each marker (total 28 mg protein). Electrophoresis at 150 V, 30 mA, 4.5 h. **11.** Gel rods of 15% PA concentration using a gel rod cassette 40,40,20 and 20 µg of each marker. Electrophoresis at 110 V, 15 mA for 4.5 h. **12.** Electrophoresis of trypsin in two normal gel slabs in a cold room. 2 mg of trypsin in each gel. Electrophoresis at 120 V, 25 mA for 3 h.



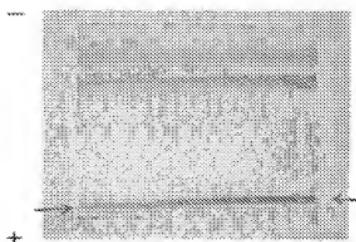
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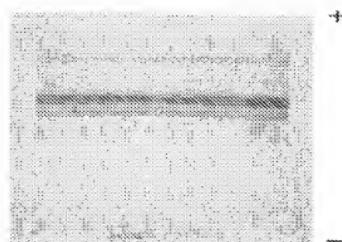
14 (a)



14 (b)



15 (a)



15 (b)

**Figures 13-15.** 13. DNA marker in 0.8% agarose gel, normal size gel 0.1  $\mu$ g of DNA marker loaded in a well. Electrophoresis at 60 V, 15 mA for 2.5 h. 14. Simultaneous forward and reverse electrophoresis of fungal broth, (a) Forward run at pH 8.9; (b) Reverse run at pH 8.9. Broth sample (5 mg protein) in the gel of each chamber. Electrophoresis at 120 V, 20 mA for 5 h. 15. Simultaneous forward and reverse electrophoresis of fungal broth (a) Forward run at pH 8.9; (b) Reverse run at pH 4.3. Broth sample (5 mg protein) in the gel of each chamber. Electrophoresis at 120 V, 20 mA for 3.5 h.

The present construction is very simple and thus allows reproduction in a poorly equipped workshop. The design will be of help to those who have only rather small resources.

It is possible that when very high current densities are used, as in a few cases, the present apparatus may not be able to solve the heat dissipation problem, unless some additional cooling arrangement is introduced. This problem can however be easily solved by sandwiching the gel cassettes or the gel between cooling plates as described by Tichy (1966).

The hollow wedge can also be used as one of the cooling plates.

### Acknowledgements

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**Q. Which 2-D gel thickness should I use?**

A. Either 1.0- or 1.5-mm-thick spacers can be used for all vertical formats. Thinner gels stain and destain more quickly and generally give background staining. Thicker gels allow easier positioning of the IPG strip on the surface of the SDS gel and have higher protein capacities. Gels are also less fragile and easier to handle.

[Click here for more information on 2-D Electrophoresis.](#)

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**Order information**

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CyDye DIGE Fluor, minimal labeling kit	5 nmol		25-8010-65	country select
DeStreak Rehydration Solution	5 x 3 ml		17-6003-19	country select
DeStreak Reagent	1 ml		17-6003-18	country select
2-D Clean-Up Kit	50 samples		80-6484-51	country select

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## Modification of commercial and custom-built slab gel electrophoresis units for two-dimensional polyacrylamide gel electrophoresis<sup>\*1</sup>

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## Abstract

Many commercial and custom-built slab gel electrophoresis units can be modified to function as two-dimensional polyacrylamide gel electrophoresis units with the insertion of Plexiglas adapters. These adapters can be made for about \$50 a pair and can be used for either temporary or permanent modification of the slab gel units. The physical dimensions of the adapters can be varied to permit great flexibility in the diameter of cylinder gels and the thickness of slab gels that can be run together. For example, proteins from 6-mm cylinder gels can be easily separated on 1-mm slab gels, which can then be dried for autoradiography.

## Article Outline

- References

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<sup>2</sup>Recipient of NIH Postdoctoral Fellowship F32-AI00158 at the time this work was done.

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## CHAPTER5: PROCEDURE 5.2

### POLYACRYLAMIDE GEL ELECTROPHORESIS

#### BACKGROUND

Running polyacrylamide gels is part of working in any modern laboratory where protein or nucleic acid samples are analyzed. Typically, when purity needs to be monitored closely, for example, when proteins are being purified for the first time, PAGE is used before and after each purification step to evaluate the success of that step. We will use it to evaluate the success of our purification by running samples on the gel from each stage of our purification. PAGE is an indispensable analytical tool for protein chemists and there is no other technique with the resolving power of PAGE that is as inexpensive and widely available.

SDS Polyacrylamide gels (SDS-PAGE) that we will be using in this procedure, are called "denaturing gels" because they contain sodium dodecyl sulfate (SDS), an ionic detergent that binds to the amino acid residues in the proteins. Due to its ionic properties, SDS confers a net negative charge on all the proteins, overcoming any intrinsic charge; in this way the proteins uniformly migrate toward the positive electrode. SDS also disrupts the secondary and tertiary structure of the proteins, essentially destroying their globular configuration and making them into linear molecules that then migrate in the electric field on the basis of their size. PAGE is a very powerful technique because even small differences in molecular weights produce distinguishable bands on a gel.

#### **REMEMBER THAT ACRYLAMIDE IS A POTENT NEUROTOXIN. ALWAYS WEAR GLOVES WHEN WORKING WITH IT.**

There are many different types and brands of electrophoresis equipment. There are also many different methods for casting gels, including commercially prepared gels that are purchased "ready to use". As you encounter these in the workplace, remember that none of these methods is necessarily better or worse although some methods are easier than others. They all accomplish the same goals.

Before you begin, make sure you understand the basic principles of SDS-PAGE as discussed in lecture. In addition, read all the instructions and watch the instructor's demonstration before attempting this procedure. Bear in mind that there is no substitute for repetition. The more gels you run, the better your gels will turn out.

You will prepare and run at least three gels. The first gel will be a practice gel. For the practice gel choose samples that are not scarce or valuable such as the commercial  $\beta$ -galactosidase and the molecular weight markers. Do not use samples from your purification until the second gel. Hopefully, you will make all the mistakes you are going to make on the first gel and won't have to waste your valuable samples.

For the second gel, you will need the following samples:

- pure  $\beta$ -galactosidase (commercial preparation)
- crude extract
- dialysate or column start (sample you loaded on your column)
- pooled samples from your column
- any other samples you wish to run

For the first and second gels, protein bands will be detected using Coomassie Blue staining. The third gel will not be stained and be used for the immunoblotting procedure, which is discussed later. Additional gels can be run for silver staining or other staining methods if time permits.

### **Preparing your samples:**

Note that the sample buffer has  $\beta$ -mercaptoethanol in it. Handle samples in the fume hood if possible.

1. Prepare your samples. Place 18  $\mu$ l of each sample in a microfuge tube and add 6  $\mu$ l of sample buffer (4x) to each sample. If you have less than 24  $\mu$ l of a sample, make up the difference with water. These instructions are based on a 10 well comb and a 1.5 mm thick gel. Note that the well volumes formed by different combs and different gel thickness accommodate different sample volumes. Refer to Table 5.1 for details.
2. Using a pushpin, poke a hole in the top of the sealed microfuge tubes containing your samples. Place the tube in a boiling water bath for 2 minutes. After removing the tubes from the boiling water, spin down any condensed water or disturbed sample in a microfuge for a few seconds. Boiling your samples in sample buffer changes your proteins into long strands, disrupting their three-dimensional structure and making all the amino acids available to bind the negatively charged detergent, SDS. Note that the sample buffer contains  $\beta$ -mercaptoethanol ( $\beta$ -ME).
3. Check to see that each well has roughly the same volume; in this case, approximately 25  $\mu$ l. Use a gel loader tip to carefully layer each sample in its well. The sample will sink to the bottom of the well, displacing the buffer. It is usually a good idea not to use the first and last wells.

Table 5.1 Sample Well Volumes for Different Combs.

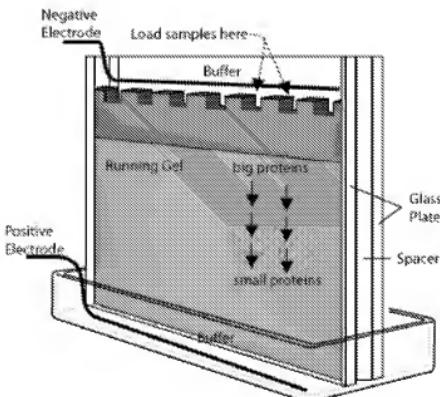
	Comb Thickness		
	0.50 mm	0.75 mm	1.5 mm
5-well comb	23 $\mu$ l	33 $\mu$ l	65 $\mu$ l
10-well comb	8 $\mu$ l	13 $\mu$ l	25 $\mu$ l
15-well comb	5 $\mu$ l	8 $\mu$ l	5 $\mu$ l

- ◆ Place the safety lid on the unit and attach the leads to the power supply.

## **LOADING THE GEL:**

1. Check for availability of all needed reagents and equipment. See Appendix A for recipes.
  - ◆ Sample buffer
  - ◆ Running buffer (Laemli buffer)
  - ◆ Staining solution or fixative (depending on visualization method)
  - ◆ Apparatus
  - ◆ Precast gels (make sure they fit with your apparatus and do not leak)
  - ◆ Power supply
2. Remove pre-cast gels from packaging and assemble in apparatus according to manufacturer directions (instructor hand-out optional here).  
Gently remove the comb. Rinse the sample wells with running buffer and drain by inverting and shaking the unit over the sink.
3. To facilitate sample loading, a 'well location decal' can be used. The decal is a clear plastic square that adheres to glass when wet. The outline of three different combs, corresponding to 5, 10 and 15 wells respectively, is marked along three separate edges of the decal. Simply wet the decal and place it against the front of the sandwich with the appropriate edge outlining the sample wells. Alternatively, you can take a marker and put a dot under each of the wells.
4. Fill the sample wells and the upper and lower buffer chambers with electrophoresis buffer (Laemli buffer).
5. Using a micropipettor (extra long tips can be used), carefully load each well with the appropriate sample. DO NOT forget to write down the contents of each well.

**SAFETY NOTE:** The electrophoresis unit is designed to prevent you from contacting the gel or buffer while voltage is applied. Never by-pass this safety feature. Also, never touch a puddle around a gel box while voltage is applied to the apparatus.



## RUNNING THE GEL

Gels may be run at either constant current or constant voltage. We are using what is known as the discontinuous (Laemmli) system because the upper and lower gel buffers are different. Constant current is usually used with a discontinuous buffer system so that the rate of electrophoretic migration will remain constant throughout the run. We have obtained good results at a constant current of running 15-20 mA. per gel. If you have two gels, you will need to double the mA. However, we have also found it convenient to run Laemmli gels at constant voltage of 90 V –125 volts using a small power supply such as the Hoeffer PS 150. (Although you do double the current if running two gels, do NOT double the volts for 2 gels. Why?) Refer to the product literature for the power supply for help. (What might happen if you run your gel at too low a voltage or amperage? What might happen if you turn it up too high?)

Electrophoresis is governed by Ohm's Law,  $V = IR$ , where the gel is the resistor and the power supply determines both the current ( $I$ ) and the voltage ( $V$ ). If the current is held constant, the voltage will increase during the run as the resistance goes up. (The resistance of the gel goes up because the ions in the gel run out reducing its conductivity.) Would you expect the resistance of a 0.5 mm thick gel to be the same as our 1.5 mm thick gels? What is the gel were twice as long?

1. Place the safety lid on the gel box unit and attach the electrode leads to the power supply. Turn on the power to the power supply and set it to the appropriate voltage or current level. Check to make sure that bubbles are forming around the electrode wires.
2. Periodically check your gel to make sure the running buffer has not leaked out of the upper buffer chamber. This is especially important when using precast gels from some

manufacturers. (What will happen if the upper chamber is empty?)

3. When the tracking dye reaches the bottom of the gel, i.e., below the surface of the lower buffer, turn off the power supply, disconnect the leads and remove the lid of the unit. Do not run the dye front off the gel.
4. Pour out the buffer by inverting the entire unit over a sink.
5. Pry open the gel sandwich. Remove the spacers and peel the gel off the plate into a tray of stain. Wetting the gel helps to loosen it from the plastic plate.
6. Rinse the lower buffer chamber and upper buffer chamber pods with distilled water after each use. Be careful not to damage the platinum wires in your electrophoresis apparatus. Be sure to clean everything carefully and put all the parts away properly.

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